FURTHER STUDIES ON THE NUTRITION OF THE FOWL NEMATODE *ASCARIDIA LINEATA* (SCHNEIDER)

by

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B. S., University of Tulsa, 1935

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1937

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INTRODUCTION

That chickens may be resistant to the viability and growth of the large roundworm, Ascaridia lineata (Schneider), was shown by Ackert and Herrick (1928) in studies on the effect of this parasite on growing chickens. These authors found that heavy infestations of this roundworm caused very deleterious effects upon young chickens, but that when the chickens were three or four months of age before becoming infested the effects were greatly minimized if not lacking altogether. Subsequent studies by Ackert, Porter and Beach (1935) gave further evidence that the resistance of growing chickens to A. lineata develops rapidly. Even two additional weeks of age in the chickens caused marked increases of resistance to the growth of these parasites.

In an effort to study the nature of the resistance of older chickens to the growth of these parasites, Ackert and Whitlock (1935) began a series of studies in which comparisons were made between the growth of A. lineata in normally fed chickens and that of A. lineata in chickens of the same age, nourished only by water and by intramuscular injections of some nutrient. Several solutions were tried. One of them, a glucose solution, gave results which made

such comparisons possible. The results of the tests indicated that A. lineata feeds to a considerable extent upon host ingesta and intestinal secretions. As these tests were preliminary in character, the present studies were undertaken with a view of making a thorough investigation of the subject.

ACKNOWLEDGMENTS

The writer wishes to express his indebtedness to Dr. J. E. Ackert for suggesting and directing this study, and to Mr. W. Malcolm Reid for his assistance in conducting the experiments.

MATERIALS AND METHODS

The Chickens

The chickens used in these experiments were White Leghorns and White Leghorn-Rhode Island Red hybrids secured and banded as day old chicks. They were raised under conditions slightly modified from those found by Herrick, Ackert and Danheim (1923) to be adequate for chickens raised in confinement.

The Normal Ration

The normal ration for the chickens consisted of corn meal (40 grams), cracked wheat (24 grams), ground oats (14 grams), meat scraps (10 grams), milk powder (5 grams), alfalfa leaf meal (5 grams), and cod liver oil (1 gram). This ration was altered slightly from time to time to correspond with that used at the Kansas State College Poultry Farm. In order to retain adequate vitamins, fresh mixes of the ingredients were made every second week. The ration was hopper fed to the chickens.

The Injected Nutrients

The nutrient solution injected into the experimental chickens was compounded from a modification of Locke's physiological solution and chemically pure dextrose. The solution consisted of 9 gm. NaCl, 0.42 gm. KCl, 0.24 gm. $CaCl_2$ and 0.1 to 0.3 gm. $NaHCO_3$ dissolved in one liter of sterile distilled water. To 75 cc. of this solution, 25 gm. of glucose were added to form the injection fluid.

The Parasite

The parasite used in these experiments was the large roundworm, Ascaridia lineata (Schneider) of chickens which inhabits the anterior portion of the small intestine. It is of very common occurrence in chickens in temperate climates. In Kansas it was present in 49 per cent of one thousand farm chickens selected at random (Ackert, 1930). In chickens less than two months of age, these worms, if present in large numbers, are very detrimental to the hosts. The effects include loss of blood, retarded growth, reduced blood sugar, increased urates, effects upon the thymus glands and increased mortality.

The nematode eggs used for parasitizing the chickens were secured from adult worms of approximately the same length. In culturing, the anterior end of the female was excised and the viscera pressed out into a sterile Petri dish. With the aid of needles, the uteri were isolated and minute openings made at different levels to permit the emission of a few eggs. Examination under the low power compound microscope disclosed the fertile eggs with their light centers (Ackert, 1931). Those portions of the uteri which had discharged fertile eggs were placed in other sterile Petri dishes and the eggs pressed out and covered with

sterile distilled water for culture. Six or eight drops of two per cent formalin were added to prevent mold and fungous growth. The best results were obtained by incubating the eggs at temperatures varying from 25° to 30° C. for a period of four to five weeks. Such cultures remained viable for an additional month or so.

Procedure

At 29 days of age the chickens were weighed and matched to assure the same ancestry and approximate weight. The chickens were then parasitized with 50 ± 5 embryonated eggs of the nematode. The eggs were counted in a drop of water on a standard microscopic slide with the aid of a low power compound microscope and mechanical stage. The dose of about 50 eggs was wiped off the slide with a piece of filter paper and placed in the chicken's gullet. After this the chickens were allowed to range free in the pens on normal rations for seven days to enable the young worms to become established.

At that time they were separated into two equal groups and half of the chickens placed in compartments where water and heat were available. These constituted the experimental group. They received no food by mouth, but at eight-hour intervals, each chicken was given intramuscular

injections of 25 per cent glucose in Locke's solution at the rate of 1 gram of glucose for each kilogram of body weight. Chickens weighing 125 grams to 225 grams were treated as weighing 200 grams; those weighing above 225 grams were treated as weighing 300 grams. This procedure was found by Whitlock (1935) to be the most advantageous. The other chickens, which constituted the control group, were allowed to range free in the pens on normal rations.

Upon the death of each experimental bird its control mate was killed and the intestines of the two flushed out by the hydraulic method of Ackert and Wolf (1929). The worms were then isolated, counted, and measured. The length of each worm was determined by having its shadow, magnified six times, thrown on the ground glass of a photographic bellows. The length of the worm, traced on onion skin paper, was determined with the aid of a milled tracing wheel. This reduced considerably the percentage of error in measuring.

EXPERIMENTS

In the first experiment 24 chickens were used. Twelve of them, the experimental group, were placed in confinement pens where they received only water *per os* and intramuscular

glucose injections. In injecting, the breast feathers were clipped, the down removed, and the area sponged with 60 per cent alcohol. With the chickens hung head downward, 0.2 cc. portions of the glucose solution were administered hypodermically into the breast until the desired amount was injected. The chicken was then returned to the confinement pen for an eight-hour period, after which the above process was repeated thrice daily.

At first, subcutaneous blood clots occurred which resulted from puncturing small blood vessels. These clots were removed by small incisions which quickly healed. In some of the experimental chickens subcutaneous edema occurred which was relieved by drainage incisions. The feces appeared watery and tinged with bile. Although the birds were active and in rather good health, they were restless due perhaps to isolation and hunger.

Late in the first evening one of the experimental chickens died. Its control was killed and the intestines of both flushed. Upon examination the experimental bird had no worms, but the control had two worms which averaged 3.6 mm. in length.

On the sixth day another experimental bird died. Its control was killed and both birds examined. The experimental chicken had 25 worms and the control, 18. The average

length of the worms from the experimental individual was 4.57 mm. and that of those from the control, 7.7 mm. The experimental birds now began to lose weight and were indifferent to their surroundings. They remained near the light, with the wings drooping and the eyes closed. After each injection the birds huddled closely to the light, withdrew their heads and ruffled their feathers, indications of temporary interference with the thermostatic balance and an increase in metabolism due to the glucose .

On the eighth day, three experimental birds died; their controls were killed, the intestines flushed and the worms isolated. The experimental chickens averaged 7.6 worms per host and the controls, 8.6. The average length of the worms from the experimental birds was 5.6 mm. as compared with 10.1 mm. for those of the controls.

The next day three of the experimental birds died and they and their controls were examined. An average of three worms was found in the experimental chickens as compared with 23 worms per fowl from the controls. In length the worms from the experimental chickens averaged 3.76 mm. and those from the controls, 15.41 mm.

The four remaining chickens of the experimental group died on the 11th day when their controls were killed. The experimental chickens averaged five worms per host and the

controls 20.3 worms per bird. The average length of the worms from the experimental birds was 5.33 mm. and that of the controls, 20.78 mm.

The results of this experiment showed that the viability and growth of the worms in the chickens were markedly affected by the lack of oral nutrition, thus supporting the previous findings of Ackert and Whitlock (1935) and indicating that host ingesta forms a portion of the diet of A. lineata. To ascertain whether or not the differences observed were due to chance variation, the experiments were resumed and continued through Experiments 2, 3 and 4, until 96 chickens had been used in the tests.

To facilitate description and analysis of the results of these four experiments the data are considered in three periods. Ackert (1931) found that the young A. lineata pass through three phases in their larval development. In the first period, the first ten days, the newly hatched larva is close to the villi but free in the intestine; in the second period, the tenth to the 18th day, the larva lies deep among the villi with the anterior end penetrating into the crypts of Lieberkühn; and in the third period, the 18th day and later, the growing worm withdraws its anterior end from the mucosa and lives free in the intestine.

Results from the First to the Tenth Day

As has been stated, the chickens after being parasitized were left free in the pens on normal rations for seven days to enable the worms to become established in the hosts. On the seventh day after parasitizing, the experimental group was confined and the injections begun as described. On the eighth day one experimental chicken died. Its control mate was killed and both birds examined with the result that two worms averaging 3.6 mm. in length were found in the control. No other individuals died during this period.

Results from the Tenth to the Eighteenth Day

On the 12th day an experimental chicken died and its control was killed. The experimental bird had seven worms and the control, two worms. The average length of the worms from the former was 3.44 mm. and that of the worms from the latter, 5.25 mm. (Table I). The examination of another experimental bird and its control the next day showed 25 worms from the experimental fowl and 18 from the control. In length the worms from the experimental chicken averaged 4.57 mm. and those from the control, 7.77 mm. Results were next available on the 14th day from another pair

TABLE I. Comparison of the numbers and lengths of *Ascaridia lineata* from glucose-injected chickens and normally fed chickens.

Age of worms in days	Glucose-injected Group				Normally Fed Group			
	Hosts examined	Total number worms	Average number worms	Average length worms mm.	Hosts examined	Total number worms	Average number worms	Average lenghh worms mm.
8	1	0	----	----	1	2	2.00	3.60
12	1	7	7.00	3.44	1	2	2.00	5.25
13	1	25	25.00	4.87	1	18	18.00	7.77
14	1	0	----	----	1	26	26.00	10.50
15	5	28	5.60	5.30	5	31	6.20	9.80
16	6	19	3.16	3.40	6	80	13.30	13.20
17	8	25	3.13	4.68	8	23	2.87	14.10
18	9	49	5.44	4.40	9	94	10.44	19.64
19	5	25	5.00	4.50	5	12	2.40	21.42
20	2	11	5.50	4.10	2	3	1.50	17.50
21	1	0	----	----	1	5	5.00	20.42
22	6	11	1.83	4.16	6	51	8.50	28.76
23	1	4	4.00	5.72	1	8	8.00	26.87
27	1	1	1.00	3.10	1	2	2.00	40.15
Total	48	205	4.27	4.46	48	367	7.44	17.47

of chickens. Worms, however, were found only in the control which had 26 A. lineata averaging 10.5 mm. in length. On the 15th day five experimental birds died and their controls were killed. The experimental birds had an average of 5.6 worms and the controls, 6.2 worms. The worms from the experimental group averaged 5.3 mm. in length while those of the control group averaged 9.8 mm. Six experimental birds died on the 16th day and their controls were sacrificed. An average of 3.16 worms was obtained from the experimental birds as compared with a 13.3 worm average from the controls. The worms from the chickens not fed by mouth grew only an average of 3.4 mm. while those from the normally fed chickens averaged 13.2 mm. On the 17th day eight experimental chickens died and their controls were killed. Upon examination the experimental birds had an average of 3.13 worms and the controls, 2.87 worms. The average length of the worms from the former was 4.68 mm. and that from the latter, 14.1 mm.

The results obtained during this period show a marked difference in the rates of growth of the A. lineata from the two groups. The worms from the experimental chickens grew very little while those from the control fowls showed a steadily increasing rate of growth. There was a decrease

in the incidence of infestation in both groups, probably due to age resistance.

Results from the Eighteenth to the Twenty-seventh Day

The results from nine experimental chickens that died on the 18th day and from their controls showed that the experimental birds had an average of 5.44 worms and the controls an average of 10.44 worms. The worms taken from the experimental group averaged 4.4 mm. while those removed from the controls averaged 19.64 mm. (Table I). Five experimental birds and their controls were examined on the 19th day. The injected chickens had an average of five worms and the controls, 2.4 worms. The length of the worms from the experimental birds averaged 4.5 mm. and of those from the control group, 21.42 mm. On the 20th day two of the experimental chickens died and their controls were killed. An average of 5.5 worms occurred in the experimental birds and 1.5 worms in the controls. In length, the worms from the experimental group averaged 4.1 mm. and those from the controls, 17.5 mm. One experimental chicken which died on the 21st day had no worms. Its control had five worms whose average length was 20.42 mm. During the 22nd day six of the experimental chickens died. An examination of them and their controls showed that the experimental birds had an average

of 1.83 worms per host as compared with 8.5 worms from the controls. The average length of the worms from the experimental birds was 4.16 mm. while that of the worms from the controls was 28.76 mm. Another experimental chicken died on the next day and its control was killed. An examination of the pair showed that the experimental bird had four worms and the control bird eight worms. The average length of the four worms was 5.72 mm. and of the eight from the control, 26.87 mm. No further evidence was obtained until the 27th day when the last experimental chicken died and its control was killed. The experimental host had one worm and the control, two worms. The one worm was 3.1 mm. long and the two worms from the control averaged 40.15 mm.

On combining the results of the four experiments it was found that the 48 chickens in the experimental group had 205 worms, an average of 4.27 worms per host, while the 48 control chickens had 357 worms with an average of 7.44 worms per host. The average length of the worms obtained from the glucose-injected chickens was 4.46 mm. as compared with an average of 17.47 mm. for the length of the worms isolated from the normally fed chickens. These results indicate that the lack of host ingesta seriously affected the viability and growth of the worms in the experimental chickens.

Graphic Analysis of the Results

As an aid in following the course of the experiments an analysis of the data on numbers of worms is illustrated in figure 1. The incidence of infestation in the glucose-injected chickens was at the highest point on the 13th day. After this time there was a sharp reduction of the numbers of worms found in each host. The incidence of parasitism in the normally fed chickens reached a peak on the 14th day after which there was a reduction in the numbers of worms. Ackert and Herrick (1928) found that as soon as the worms began to withdraw from the mucosa they were rapidly eliminated. It would thus appear that the worms in the glucose-injected chickens began their withdrawal into the lumen about the 14th day and those in the control group, about the 15th day. Since the elimination of the A. lineata was more marked in the group that received no food by mouth than in the control group it seems evident that this greater decline in numbers of worms in the experimental chickens was due to the lack of host ingesta.

To compare more readily the growth rates of the A. lineata from the chickens nourished intramuscularly with those of the worms from chickens fed normally, the data on lengths of the A. lineata are presented graphically in figure 2.

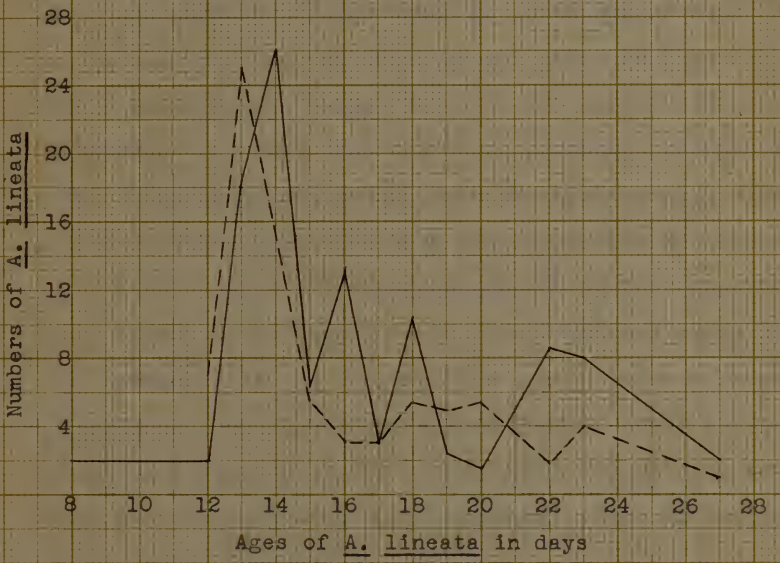

Figure 1. Comparison of numbers of A. lineata from glucose-injected and normally fed chickens.
———A. lineata from normally fed chickens
------A. lineata from glucose injected chickens

From this figure it is evident that the worms in the experimental group grew slightly until the 15th day of parasitism. After that time there was a reduction in the lengths of the worms obtained, with one slight exception on the 23rd day. From the flatness of the curve it would appear that the worms attained most of their growth prior to the eighth day, but exhausted any reserve material for growth before the 15th day. The fact that the worms obtained thd 23rd day were longer than any others from the experimental chickens would indicate that this host was an especially susceptible individual. That all other conditions of the experiment were normal is evidenced by the fact that the worms from the control chickens followed the growth curve of normal A. lineata as demonstrated by Ackert (1931). Thus the evidence as presented by the growth curves leaves no doubt that the host ingesta is an important factor in the normal growth of A. lineata.

The Bile as a Factor

In all of the experiments post-mortem examinations revealed marked contrasts between the sizes of the gall bladders of the experimental chickens and those of the control chickens. While the gall bladders of the controls were of normal size, those of the glucose-injected chickens always

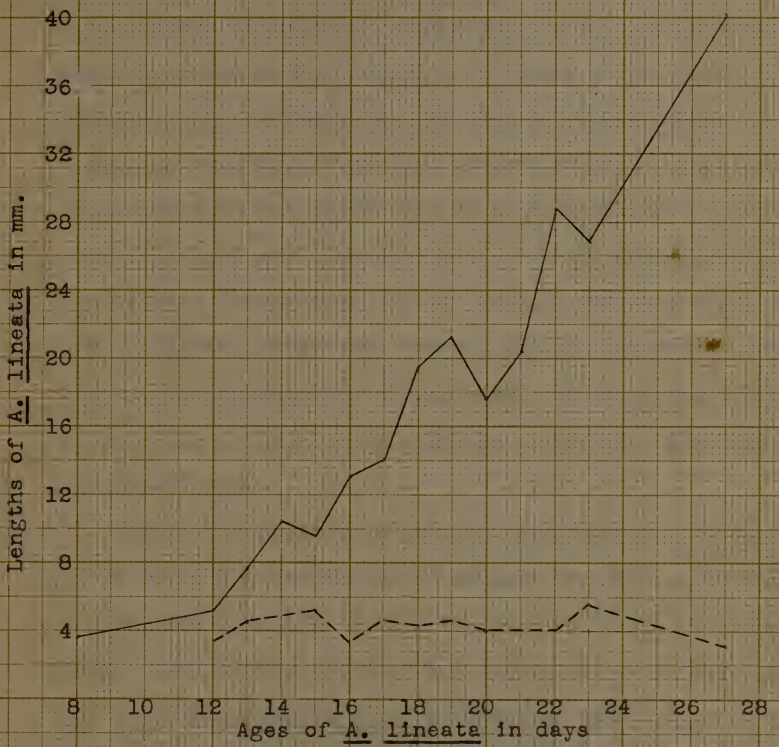

Figure 2. Comparison of lengths of A. lineata from glucose-injected and normally fed chickens.
——— A. lineata from normally fed chickens
- - - - - A. lineata from glucose-injected chickens

were greatly distended. Moreover, in the latter birds, there appeared to be a slow, but almost continuous, flow of bile as shown by the greenish tinged evacuations. As this unchanged bile might serve as a favorable, or as an unfavorable factor in the lives of the A. lineata in the injected chickens, an experiment was run in which the bile was diverted from the portion of the small intestine that the worms inhabit. This was done by making a surgical anastomosis of the duodenum to the ileum. The duodenum posterior to the bile ducts, was ligated to force the bile through the anastomosis.

After the chickens had been parasitized the usual seven days, the anastomosis was made and the experiment continued. On the 14th day the chicken with the anastomosis died. Seven worms were isolated from this bird and measured. The worms averaged 3.8 mm. in length. As no data on A. lineata from the regularly injected chickens are available on the 14th day a comparison was made with the averages of the worms recovered from the glucose-injected birds examined on the 13th and 15th days. The anastomosed individual had somewhat less than the average for the 13th day, but slightly more worms than those obtained on the 15th day. In length, the worms were well within the limits of variation of those of the glucose-injected group. From these compar-

isons it was inferred that the bile had neither an inhibitory nor a nutritive effect upon these worms. No other intestinal secretions were studied.

DISCUSSION

Little study has been made of the nutritive needs of the nematode Ascaridia lineata (Schneider), but some information is available on the Ascaridae, to which, according to Eisenbrandt (1936), A. lineata shows close relationship. The general physiology of the Ascaridae was reviewed by Winterstein in 1911 and more recently by McCoy (1935). The nutritive needs of the Ascaridae were studied by Flury (1912) who concluded that those of the Ascaridae were almost the same as the nutritive needs of other animals, since the qualitative body analysis was approximately the same. Archer and Peterson (1930) and Li (1933 a and b) in studies of the feeding habits of the Ascaridae found that the nematodes were stimulated to feed by the presence of solid particles in the host ingesta, but that in the absence of these particles the worms did not feed. These observations appear to be confirmed by the present experiments. In figure 2 the growth curve of the A. lineata from the glucose-injected chickens shows that in the absence of host ingesta the worms did not grow, especially after the

15th day. Their growth prior to that time may find explanation in the work of Giovannola (1936). This investigator, in an analysis of the energy and food reserves of nematodes found that during the first larval stage a food reserve was built up that was sufficient to carry the larva through a period of lessened nutrition. A similar situation prevailed in the present experiments. The newly hatched larvae of A. lineata had normal food during the first seven days of the experiments, affording opportunity for the storage of reserve food from which to draw for some time. This reserve appeared to become exhausted in the A. lineata about the 15th day as no consistent growth in length was observed after that time (fig. 2).

The growth of the A. lineata in the injected birds from the seventh to the 15th day might be attributed to tissue feeding, as the larvae are partially imbedded in the intestinal mucosa. But if this were a factor the young A. lineata should have continued growing through the 18th day when the last of the larvae withdraw their anterior ends from the mucosa and live free in the intestinal lumen. Furthermore, Ackert (1935), who introduced live A. lineata into the body cavities of chickens where tissue was available, found the worms unable to thrive. It, therefore, appears that the fowl nematode, A. lineata is neither able

to feed upon tissue nor to obtain its nourishment from the intestinal mucosa as was suggested by Garin (1913) for such worms.

SUMMARY

1. In studies upon the nutrition of the fowl nematode, Ascaridia lineata (Schneider) comparisons were made upon the numbers and lengths of A. lineata from chickens nourished only by intramuscular glucose injections and from chickens of the same age that were fed normally. Injections of 25 per cent glucose in Locke's solution of eight-hour intervals were the most successful in prolonging the lives of the injected fowls.

2. The results from 96 chickens showed that the worms thrived better in normally fed chickens than in those nourished only by glucose injections. The average number of worms from the glucose-injected group was 4.27 as compared with an average of 7.44 worms from the naturally fed group. As to length, those from the injected group averaged 4.46 mm. and the worms from the controls, 17.47 mm. Furthermore, the A. lineata from the controls made a normal growth while those from the glucose-injected chickens made but little growth.

3. The lowered incidence of infestation and the smaller

size of the worms in the experimental group appears to have been due to starvation from lack of host ingesta.

4. By anastomosing the duodenum to the ileum the bile was diverted from the worms' habitat; the results of the test indicated that the unchanged bile in the injected chickens was neither a positive nor a negative factor in the growth of A. lineata.

5. The results of these studies, and of others upon the Ascaridae, to which A. lineata are related serologically indicate that the method of feeding, nutritional needs and qualitative body analysis of such nematodes do not differ markedly from those of other animals.

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